By way of the present amendment, claims 1 to 7 are canceled, and new claims 8 to 19 are added. Claims 8, 12, and 13 are independent claims.

In the Office Action, the Examiner rejected claims 1 to 7 under 35 U.S.C. §§ 101, 102, 103, and 112. These rejections are rendered moot by way of the present amendment, canceling claims 1 to 7, although the prior art cited against the claims is discussed in relation to the new claims.

The Examiner cited Immunobiology, p. 21 - 30 (2000) ("Sha"), and Promega Technical Bulletin 206, rev. 7/1999 ("Promega") against the claims. The Sha reference is discussed first. Sha discloses the use of a plasmid/liposome mixture to induce mucosal immunity. Under this approach, the plasmid is mixed with a commercially available liposome made from Dosper (materials and methods, p. 22, lines 8 to 10). The plasmid formed a complex with the liposomes, and the complex is a DNA/liposome mixture.

In contrast, the present invention as presently claimed relates to the use of a liposome-encapsulated plasmid DNA where the plasmid DNA is completely encapsulated within liposomes. The liposome formulation, the method of preparing the formulation, and the resulting structure of the plasmid in liposomes are fundamentally different. The present invention describes the use of plasmid DNA which is entirely encapsulated in liposomes, rather than a plasmid DNA complex as disclosed by Sha. Thus, Sha does not teach or suggest the presently claimed invention.

Further, the approach taught by Sha is used to induce mucosal immunity in the respiratory tract, but is revealed to be ineffective in protecting mice against the influenza virus (results, p. 26, line 1 to 6, last paragraph). In contrast, the present invention as claimed involves the use of liposome-encapsulated plasmid DNA to protect against a lethal infection of the influenza virus. The present application provides unambiguous scientific evidence that liposome-encapsulated DNA vaccine provides 100% protection to mice against an influenza virus infection (p. 14, lines 5 to 14; Figs. 3 to 4). The disparity between the present application and the Sha patent reveal that the respective approaches are entirely different. A person of ordinary skill in the art would not, from reviewing Sha, determine that encapsulation of plasmid DNA within liposomes is critically important in offering influenza virus protection, but would

instead be only informed as to the success or lack thereof related to a liposome/DNA complex.

The Promega reference fails to compensate for the deficiency of Sha. Promega is cited only for its alleged teachings of the use of a pCI plasmid with a CMV promoter to clone the HA gene. Since none of the cited prior art teaches or suggest the presently claimed invention, including the features discussed above, it is respectfully requested that the presently presented claims be allowed.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue.

Dated: March 17, 2003

Respectfully submitted,

By_____
David K. Benson
Registration No.: 42,314
RADER, FISHMAN & GRAUER PLLC
1233 20th Street, N.W.
Suite 501
Washington, DC 20036
(703) 955-3750
Attorneys for Applicant

Version With Markings to Show Changes Made

8. (new) A deoxyribonucleic acid (DNA) vaccine comprising a liposome-encapsulated plasmid containing a gene encoding for hemagglutinin protein.

9. (new) The vaccine as claimed in Claim 8 deliverable to a respiratory tract using intranasal administration and/or by aerosol inhalation.

10. (new) Use of the vaccine of claim 8 for preventing and/or treating influenza virus infection.

11. (new) Use of the vaccine of claim 8 for eliciting long-lasting protective antiviral immune responses against influenza viruses.

12. (new) A plasmid vector construct pCI-HA10 comprising a gene encoding for hemagglutinin protein and capable of expressing said hemagglutinin protein in a host.

13. (new) A method for constructing a plasmid pCI-HA10 comprising the following steps:

(1) amplifying hemagglutinin gene from viral and mRNA with PCR;

(2) inserting and ligating the hemagglutinin gene into a pCI vector;

(3) transforming the resulting vector into competent E.coli DH5α cells;

(4) transcribing and translating of pCI-HA10; and

(5) preparing and purifying pCI-HA10 by bulk preparation method.

14. (new) A liposome formulation for encapsulating the plasmid pCI-HA10 of claim 12 comprising of 7% 1,2 dioleoyl-3-dimethylammonium chloride (DODAC), 78% 1,2-dioleoyl-sn-glycrerol-3-phospho-ethanolamine (DOPE) and 15% polyethylene glycol C8 ceramide ($PEG_{2000}C_8CER$).

15. (new) A method for encapsulating the plasmid pCI-HA10 of claim 12 into liposomes comprising the following steps:

(1) preparing 7% DODAC, 78% DOPE, and 15% $PEG_{2000}C_8CER$ at 10mg/ml concentrations to form a lipid film at 50 °C for 2h under vacuum;

(2) incubating the lipid film at 50 °C for 2h under vacuum;

(3) reconstituting the lipid film with distilled water and 1M β-octylglucanopyranoside detergent at 20% of the total preparation volume;

(4) adding the plasmid DNA to the lipid film at a concentration of 400 μg DNA/ml of 10 mg/ml;

(5) transferring the reconstituted preparation into dialysis tubing and dialyzing in 1X HEPES buffer solution (150 mM NaCI, 20 mM Hepes, pH 7.4) at 23 °C for 15 h; and

(6) removing the free, non-encapsulate DNA from encapsulated DNA on a DEAE Sepharose CL-6B anion exchange column.

16. (new) A liposome-encapsulated pCI-HA10 constructed in accordance with the method of claim 15.

17. (new) A method of delivering the liposome-encapsulated pCI-HA10 of claim 16 to a respiratory tract using intranasal administration, and/or aerosol inhalation to eliciting protective antiviral immune responses to influenza viruses.

18. (new) A method for preventing and/or treating influenza virus infection, comprising administering to a patient in need thereof a pharmaceutically effective amount of the vaccine of claim 8.

19. (new) A method for eliciting long-lasting protective antiviral immune responses against influenza viruses, comprising administering to a patient in need thereof a pharmaceutically effective amount of the vaccine of claim 8.